Amendments to the Specification

Please delete the paragraph at line 1 on page 1 requested in Applicants' December 8, 2003 Preliminary Amendment.

Please insert the following heading and paragraph line 2 on page 1:

PRIORITY CLAIM TO RELATED PATENT APPLICATIONS

This patent claims priority as a divisional to U.S. Patent No. 6,682,745 (corresponding to U.S. Patent Application No. 09/492,206, filed January 27, 2000). U.S. Patent No. 6,682,745, in turn, claims priority as a continuation-in-part of U.S. Patent No. 6,120,775 (corresponding to U.S. Patent Application No. 09/123,735, filed July 28, 1998), which, in turn, claims priority to European Patent Application Nos. 97202365 (filed July 29, 1997) and 97202925 (filed September 24, 1997). U.S. Patent No. 6,682,745 also claims priority to European Patent Application No. 99200202 (filed January 26, 1999).

Please amend the paragraph bridging lines 3-13 on page 1 in the following manner, which includes: (1) splitting the paragraph into two paragraphs, and (2) adding the "field of the invention" and "background of the invention" headings:

FIELD OF THE INVENTION

The present invention relates to the use of bacteria for the manufacture of vaccines.

BACKGROUND OF THE INVENTION

Vaccination has been proven through the years to be a very efficient method for the prevention of diseases caused by many different bacteria. Vaccines have the advantage, contrary to e.g. antibiotic or pharmacochemical therapies, that they are preventing disease rather than curing it. In many fields, e.g. the field of animal husbandry, vaccination is a standard routine. Usually, all animals in a group are vaccinated as a precautionary measure, in order to prevent disease, whereas in practice often only a few animals would have become infected if no vaccine had been given. This explains why for most commonly used vaccines

adverse local reactions due to vaccination are not acceptable: it is not acceptable to cause (severe) physical stress in many animals to prevent a (mild) disease in few.

Please insert the following heading between lines 19 and 20 on page 1:

SUMMARY OF THE INVENTION

Please insert the following heading between lines 24 and 25 on page 1:

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Please amend the paragraph bridging lines 4-16 on page 2 in the following manner:

Of these routes, intramuscular vaccination is in many cases the preferred application route. This is due to the fact that the vaccine, possibly mixed with an adjuvant, is only slowly released from the site of injection. Thus, the immune system is continuously triggered for a relatively long time with an immunogenic dose of the vaccine. This way of administration ensures an adequate immune response. The disadvantage, however, is [[,]] that many bacterial IM administered vaccines cause large abscesses at the site of injection. These abscesses may stay there from days to months. In those cases in which a live attenuated bacterium must behave relatively virulent in order to trigger an adequate immune response, the bacterium often replicates at the injection site to such a level that the abscess even bursts. Large intramuscular or skin [[-]] abscesses are clearly [[an]] unacceptable ~~side-effect~~ side-effects of vaccination with bacterial live attenuated strains, but unavoidable if further attenuation spoils the immunogenic potential of the bacterium. This causes the dilemma mentioned above, for which the invention offers a solution.

Please amend the paragraph bridging lines 7-18 on page 4 in the following manner:

Streptococcus equi, the cause of "Strangles". This disease causes abscesses of lymph nodes of the head and neck and systemic infections. The swelling of the lymph nodes causes the horses to ~~be suffocated~~ suffocate. No reliable vaccine without adverse local reactions is

known so far [[,]] for *Streptococcus equi* or for *Streptococcus zooepidemicus*, causing respiratory tract infections and pneumonia, opportunistic infections and abortion in horses; [[,]] *Rhodococcus equi*, causing bronchopneumonia with abscesses and intestinal abscesses; [[,]] *Corynebacterium pseudotuberculosis*, causing pectoral abscesses and ulcerative lymphangitis; [[,]] *Pseudomonas mallei*, causing: "Glanders", a disease characterised by pyogranulomatous inflammations, nodular lesions in lung and ulcerative and nodular lesions in skin and respiratory mucosa; [[,]] *Actinobacillus equili*, a well-known cause of neonatal death, abortion in mares, stillbirth and foal septicaemia; and finally *Pasteurella multocida*, causing respiratory tract infections in horses.

Please amend the paragraph bridging lines 19-21 on page 4 in the following manner:

Horses have in many cases both a high emotional and economical value to their owners. [[,]] Especially in the field of thoroughbreds, it would be unacceptable to have horses suffering from abscesses after vaccination.

Please amend the paragraph bridging lines 22-24 on page 4 in the following manner:

Therefore, in a more preferred form of the invention, the use relates to a use where the live attenuated bacterium is an attenuated form of a horse pathogenic bacterium.

Please amend the paragraph bridging lines 5-11 on page 5 in the following manner:

Actinomyces pyogenes, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus uberis*, *Noccardia asteroides*, *Corynebacterium bovis*, *Mycoplasma bovis*, and *Mycobacterium bovis*, all well-established causes of bovine mastitis; [[,]] *Escherichia coli*, causing both bovine mastitis and diarrhoea; [[,]] *Pasteurella haemolytica* and *P. multocida*, both causing pneumonia and septicaemia; [[,]] *Brucella abortus*, causing abortion; [[,]] *Salmonella dublin* and *S. typhimurium*, causing diarrhoea, pneumonia, and systemic infections; and finally *Leptospira hardjo* as a cause of urinary tract infections.

Please amend the paragraph bridging lines 15-23 on page 5 in the following manner:

Streptococcus suis causing polyserositis, *Staphylococcus aureus* causing exudative epidermitis, *Actinobacillus pleurnopneumoniae* causing pleuropneumonia, *Pasteurella multocida* causing atrophic rhinitis and pneumonia, *Bordetella bronchiseptica* also causing atrophic rhinitis and pneumonia, *Escherichia coli* causing diarrhoea and edema disease, *Clostridium perfringens* as a cause of diarrhoea and septicaemia, *Salmonella cholerasuis* also a known cause of diarrhoea, *Haemophilus parasuis* also known as the cause of "Glassers disease", *Erysipelothrix rhusiopathiae* causing a disease known as "Erysipelas", *Mycoplasma hyopneumoniae* causing pneumonia, *Serpulina hyodysentriae* as a cause of diarrhoea, and *Leptospira pomona* that causes ~~gives~~ abortion.

Please amend the paragraph bridging lines 27-29 on page 5 in the following manner:

Staphylococcus aureus, pyoderma; [[,]] *Streptococcus pneumoniae*, septicaemia; *Bordetella bronchiseptica*, tracheobronchitis; [[,]] *Eschenchia coli*, diarrhoea; and *Leptospira canicola* and *icterohaemorrhagiae*, general and urinary tract infections.

Please amend the paragraph bridging lines 1-4 on page 6 in the following manner:

The manufactured vaccines comprise at least an "immunogenically effective" amount of a live attenuated bacterium. Immunogenically effective means that the amount of live attenuated bacterium administered at vaccination is sufficient to induce in the host an effective immune response to virulent forms of the bacterium.

Please amend the paragraph bridging lines 5-8 on page 6 in the following manner:

The useful dosage to be administered will vary depending on the [[of]] age, weight, and type of mammal to be vaccinated, as well as [[and]] the type of pathogen against which

vaccination is sought. The vaccine may comprise any dose of bacteria sufficient to evoke an immune response. Doses ranging between, **c.g.,** 10^3 and 10^{10} bacteria are **[[e.g.]]** very suitable doses.

Please amend the paragraph bridging lines 11-16 on page 7 in the following manner:

After a 2-**week [[weeks]]** acclimatisation period, 5 horses were vaccinated submucosally in the lip with strain TW 928 deletion mutant. Vaccination was done at 2 spots in the upper lip and 2 spots **in the** lower lip. A needle was used that was provided with a disc of about 1 centimetre diameter, attached at right angles to the needle, and located at about 2.5 ~~millimetres~~ **millimeters** from the tip of the needle. This prevented the tip of the needle **from entering** ~~to enter~~ the submucosa **[[for]]** more than about 2 ~~millimetre~~ **millimeters**.

Please amend the paragraph bridging lines 23-28 on page 7 in the following manner:

At 4 weeks after priming vaccination, the vaccinates were boosted as described above with the same amount of bacteria at similar vaccination sites. At 2 weeks after booster vaccination, all horses were challenged intranasally with 7.7.times.10.sup.8 CFU of the challenge strain S. equi strain Arnica in a 2 ml volume. After vaccination the horses were observed for any systemic or local reactions and after challenge, the horses were examined for clinical signs of strangles or any other abnormality.

Please amend the paragraph bridging lines 2-6 on page 8 in the following manner:

Horses subjected to intramuscular vaccination in the neck developed large abscesses that reached diameters ranging between 10 and 30 **centimeters** ~~centimetres~~ within weeks after vaccination. These abscesses were persistent and kept growing until they burst. Horses subjected to submucosal vaccination appeared in a good condition and had a normal appetite, and no significant further systemic reactions were observed.

Please amend the paragraph bridging lines 7-11 on page 8 in the following manner:

After submucosal priming and boosting with the 928 deletion mutant, only small and transient local reactions were found. Most reactions had disappeared at 3 weeks after priming vaccination and at 2 weeks after booster vaccination. The same minor local reactions, but to an even lesser ~~extend~~ extent, were observed after both vaccinations with the 928/sls double mutant.

Please amend the paragraph bridging lines 16-17 on page 8 in the following manner:

- full protection can be obtained with suitable vaccine strains regardless the site of administration, [[;]] intramuscularly, or submucosally.

Please amend the paragraph bridging lines 6-8 on page 9 in the following manner:

The intramuscularly vaccinated horses developed large abscesses from the fourth day after vaccination, that grew to an average size, at ten days after vaccination, of about 20 centimeters ~~centimetres~~ diameter. These abscesses were persistent.

Please amend the paragraph bridging lines 9-11 on page 9 in the following manner:

The submucosally vaccinated horses only developed minor abscesses with an average size of 2.5 ~~centimetres~~ centimeters, beginning at day 5 after vaccination. The abscesses completely disappeared after six days, leaving no traces behind.

Please amend the paragraph bridging lines 25-27 on page 9 in the following manner:

In the submucosally vaccinated animals, small abscesses developed after three days, reaching an average diameter [[size]] of about 3.5 ~~centimetres diameter~~ centimeters.

These abscesses decreased in size after a few days.

Please amend the paragraph bridging lines 28-30 on page 9 in the following manner:

In the two cows vaccinated intramuscularly in the neck with the same dose, large and more persistent abscesses developed after three days, reaching a diameter of **between 9** ~~**between9**~~ and 14 ~~**centimetres**~~ **centimeters**.